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(54) Title: RECOMBINANT KAT ENZYME AND PROCESS FOR ITS PREPARATION

(57) Abstract

Disclosed are isolated DNAs encoding a kynurenine aminotransferase selected from the group consisting of: (a) isolated DNA sequences which encode rat KAT; (b) an isolated DNA sequence which hybridizes to isolated DNA sequences of (a) above and which encodes a mammalian KAT enzyme; and (c) an isolated DNA sequence differing from the isolated DNA sequences of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a KAT enzyme. Vectors and host cells containing the same, oligonucleotide probes for identifying kynurenine aminotransferase, and isolated and purified kynurenine aminotransferase are also disclosed.

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RECOMBINANT KAT ENZYME AND PROCESS FOR ITS PREPARATION

FIELD OF THE INVENTION

The present invention relates to DNA sequences that code for kynurenine aminotransferase.

BACKGROUND OF THE INVENTION

The enzyme kynurenine aminotransferase (known in the art as KAT) catalyzes the biosynthesis of kynurenic acid (KYNA) from kynurenine (KYN) and is singularly responsible for the regulation of extracellular KYNA concentrations in the brain (J. Neurochem., 57:533-540 (1991)).

KYNA is an effective excitatory amino acid (EAA) receptor antagonist with a particularly high affinity to the glycine modulatory site of the N-methyl-D-aspartate (NMDA) receptor complex (J. Neurochem., 52:1319-1328 (1989)). As a naturally occurring brain metabolite (J. Neurochem., 51:177-180 (1988); and Brain Res., 454:164-169 (1988)), KYNA probably serves as a negative endogenous modulator of cerebral glutamatergic function (Ann. N.Y. Acad. Sci., 648:140-153 (1992)).

EAA receptors and in particular NMDA receptors are known to play a central role in the function of the mammalian brain (Watkins et al, In: The NMDA Receptor, page 242, (1989), Eds., Oxford University Press, Oxford). For example, NMDA receptor activation is essential for cognitive processes, such as, for example, learning and memory (Watkins et al, In: The NMDA Receptor, Eds., pages 137-151, (1989), Oxford University press, Oxford) and for brain development (Trends Pharmacol. Sci., 11:290-296 (1990)).

It follows that a reduction in NMDA receptor function will have detrimental consequences for brain

physiology and, consequently, for the entire organism. For example, the decline in the number of NMDA receptors which occurs in the aged brain (Synapse, 6:343-388 (1990)) is likely associated with age-related disorders of cognitive functions.

In the brain, KYNA concentrations and the activity of KYNA's biosynthetic enzyme KAT show a remarkable increase with age (Brain Res., 558:1-5, (1992); and Neurosci. Lett., 94:145-150 (1988)). KAT inhibitors, by providing an increase of the glutamatergic tone at the NMDA receptor, could therefore be particularly useful in situations where NMDA receptor function is insufficient and/or KAT activity and KYNA levels are abnormally enhanced. Hence they could be particularly useful in the treatment of the pathological consequences associated with the aging processes in the brain which are, for example, cognitive disorders including, e.g., attentional and memory deficits and vigilance impairments in the elderly.

KAT inhibitors may also be useful in the treatment of perinatal brain disorders which may be related to irregularities in the characteristic region specific pattern of postnatal KAT development (Baran et al, Dev. Brain Res., 74:283-286 (1993)).

In subcellular fractionation studies KAT activity was recovered either in the cytosol and in mitochondria (J. Neurochem., *supra*).

Most nuclear-encoded precursors of mitochondrial proteins contain amino-terminal presequences (Pfanner et al, In: Current Topics in Bioenergetics, 15:177-219 (1987); Lee Ed., New York Academic Press; and Nicholson et al, In: Protein Transfer and Organelle Biogenesis, Das and Robins Eds., New York Academic Press (1988)). These presequences are required for the precursor to enter the mitochondrial matrix, where they are proteolytically removed (Hurt et al, FEBS Lett., 178:306 (1984);

Horwich et al, EMBO J., 4:1129 (1985). This cleavage is not essential for completing import but is necessary for further assembly of the newly imported polypeptides into functional complexes (Zwizinski et al, J. Biol. Chem., 258:13340 (1983); Lewin et al, J. Biol. Chem., 258:6750 (1983); Ou et al, J. Biochem., 100:1287 (1986)). Precursor targeting sequences differ considerably in their structures. One of the few common themes is the high content of positively charged amino acids and of hydroxylated amino acids. Presequences may form an amphipathic structure in the form of either a-helices or b-sheets (von Heijne et al, EMBO J., 5:1335 (1986); Roise et al, EMBO J., 5:1327 (1986); and Vassarotti et al, EMBO J., 6:705 (1987)). Despite the large variability of the sequences of mitochondrial leader peptides, relatively minor alterations of the presequence can prevent cleavage by the processing peptidase (Hurt et al, J. Biol. Chem., 262:1420 (1987)). This suggests that distinct, but up to now undefined, structural elements are required for cleavage. Similarly, the cleavage sites show wide variation among different precursors of a single organism and among precursors of different organisms.

Interestingly, using the protein algoritm described by Gavel et al (Protein Engineering, 4:33-37 (1990)), a potential mitochondrial transit peptide is predicted either in position 1 to 24 of the deduced protein of cDNA-2 and in position 1 to 44 of the deduced protein of cDNA-3 disclosed in the present invention (see Figures 3-4 and Example 3).

Recently Perry et al (Mol. Pharm., 43:660-665 (1993)) reported the cloning of a cDNA coding for rat kidney cytosolic cysteine conjugate ß-lyase. When the cDNA was inserted into the expression vector pVS1000 and

transfected into COS-1 tissue culture cells, a 7-10 fold increase in cytosolic ß-lyase and glutamine transaminase K activities were detected.

The deduced amino acid sequence of rat ß-lyase is identical to the deduced amino acid sequence of cDNA-1 (rat KAT) except for two residues (see Figure 2). Moreover the existence of cDNA-2 and cDNA-3 was not reported by Perry et al (Mol. Pharm., *supra*).

Even more recently Perry et al (FEBS Lett., 360:277-280 (1995)) reported the cloning of a cDNA for human kidney cysteine conjugate beta-lyase whose sequence is identical to the sequence of the human KAT described in the present patent application.

Whereas the identity with cysteine conjugate ß-lyase and glutamine transaminase K is well documented (Abraham et al, Analytical Biochem., 197:421-427 (1991)), there are no reports indicating identity of kynurenine transaminase neither with ß-lyase nor with glutamine transaminase K.

SUMMARY OF THE INVENTION

We now report the cloning of mammalian kynurenine aminotransferases.

A first aspect of the present invention are isolated DNA sequences encoding a KAT enzyme selected from the group consisting of: (a) isolated DNA sequences which encode rat KAT; (b) an isolated DNA sequence which hybridizes to isolated DNA sequences of (a) above and which encodes a mammalian KAT enzyme; and (c) an isolated DNA sequence differing from the isolated DNA sequences of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a KAT enzyme.

A second aspect of the present invention are vectors comprising a cloned DNA sequence as given above.

A third aspect of the present invention are host cells transformed with a vector as given above.

A fourth aspect of the present invention is an oligonucleotide probe capable of selectively hybridizing to a DNA comprising a portion of a gene coding for a KAT enzyme.

A fifth aspect of the present invention is isolated and purified KAT enzyme which is coded for by a DNA sequence selected from the group consisting of: (a) isolated DNA sequences which encode rat KAT; (b) isolated DNA sequence which hybridizes to isolated DNA sequence of (a) above and which encodes a mammalian KAT enzyme; and (c) an isolated DNA sequence differing from the isolated DNA sequences of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a KAT enzyme.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Partial amino acid sequence of rat KAT: N-terminus of mature KAT (SEQ ID NO:14), a CNBr fragment (SEQ ID NO:15), tryptic fragment 112 of KAT (SEQ ID NO: 16) and tryptic fragment 130 of KAT (SEQ ID NO:17).

Figures 2A-2C nucleotide sequence and deduced amino acid sequence of rat KAT (cDNA-1) (SEQ ID NO:18). The putative pyridoxal phosphate binding site, Ser-Ala-Gly-Lys-Ser-Phe, is underlined. Triplets differing from rat ß-lyase cDNA (Perry et al, supra) are boxed.

Figures 3A-3D nucleotide sequence and deduced amino acid sequences of rat KAT (cDNA-2) (SEQ ID

NO:19). Two proteins can be synthesized: one starting from

nucleotide 619 and including a putative mitochondrial targeting peptide, the other beginning at the same ATG starting codon as in the case of cDNA-1. The putative pyridoxal phosphate binding site, Ser-Ala-Gly-Lys-Ser-Phe, is underlined. Triplets differing from rat ß-lyase cDNA (Perry et al, supra) are boxed.

Figures 4A-4D nucleotide sequence and deduced amino acid sequences of rat KAT (cDNA-3) (SEQ ID NO:5). The sequence of cDNA-3 is identical to that of cDNA-1 except for an insertion of 208 base pairs in the 5'-untranslated region. The insertion creates an additional stretch of 34 amino acids in frame with the cDNA-1 deduced protein sequence. The insertion of these 208 base pairs occurs between nucleotide 237 and 238 of the cDNA-1 sequence.

Figures 5A and 5B cytosolic enzyme activities in transfected COS-1 cells: 5A, glutamine transaminase K activity; 5B, kynurenine transaminasae activity. Sense: pSVL-KAT trnsfected COS-1 cells were cDNA-1 is in the sense orientation. Antisense: pSVL-KAT transfected COS-1 cells were cDNA-1 is in reverse orientation. Each value is the mean of three separate experiments.

Figure 6 Partial amino acid sequence of human KAT I: tryptic fragments F11 (SEQ ID NO:2); F13 (SEQ ID NO:3); and F14 (SEQ ID NO:4) of the human KAT I.

Figures 7A-7C nucleotide sequence and deduced amino acid sequence of human KAT I (SEQ ID NO:1).

DETAILED DESCRIPTION OF THE INVENTION

Amino acid sequences disclosed herein are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence. Nucleotide sequences are

presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code.

The kynurenine aminotransferase enzyme of the present invention includes proteins homologous to, and having essentially the same biological properties as, the protein coded for by the nucleotide sequences herein disclosed. This definition is intended to encompass natural allelic variants of KAT sequence.

Cloned genes of the present invention may code for KAT of any species of origin, but preferably code for enzymes of mammalian origin. Thus, DNA sequences which hybridize to the sequences given in Figures 2A-2C (SEQ ID NO:18), 3A-3D (SEQ ID NO:19), 4A-4D (SEQ ID NO:5) and 7A-7C (SEQ ID NO:1) and which code for expression of KAT are also an aspect of this invention. Conditions which will permit other DNA sequences which code for expression of KAT to hybridize to the sequences given in Figures 2A-2C (SEQ ID NO:18), 3A-3D (SEQ ID NO:19), 4A-4D (SEQ ID NO:5) and 7A-7C (SEQ ID NO:1) can be determined in a routine manner. Further, DNA sequences which code for polypeptides coded for by the sequences given in Figures 2A-2C (SEQ ID NO:18), 3A-3D (SEQ ID NO:19), 4A-4D (SEQ ID NO:5) and 7A-7C (SEQ ID NO:1) or sequences which hybridize thereto and code for a KAT enzyme, but which differ in codon sequence from these due to degenerancy of the genetic code, are also an aspect of this invention. The degenerancy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is well known in the literature. See, e.g., Toole et al, U.S. Patent 4,757,006 at column 2, Table 1.

DNA which encodes the KAT enzyme may be obtained by a variety of means well known to the expert in the art and disclosed by, for example, Maniatis et al, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1989).

For example, DNA which encodes the KAT enzyme may be obtained by screening of mRNA or genomic DNA with oligonucleotide probes generated from the KAT enzyme gene sequence information provided herein. Probes may be labeled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known procedures and used in conventional hybridization assays, as described by, for example, Maniatis et al, *supra*.

KAT gene sequences may alternatively be recovered by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers described herein or with oligonucleotide primers being produced from the KAT enzyme sequences provided herein. See Mullis et al, U.S. Patent 4,683,195; and Mullis, U.S. Patent 4,683,202. The PCR reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotide probes to serve as primers for the template-dependent, polymerase mediated replication of a desired nucleic acid molecule.

The recombinant DNA molecules of the present invention can be produced through any of a variety of means well known to the expert in the art and

disclosed by, for example, Maniatis et al, *supra*. In order to replicate the KAT enzyme DNA sequences, these must be cloned in an appropriate vector. A vector is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding the KAT enzyme and/or to express DNA which encodes the KAT enzyme. An expression vector is a replicable DNA construct in which a DNA sequence encoding the KAT enzyme is operably linked to suitable control sequences capable of effecting the expression of the KAT enzyme in a suitable host. DNA regions are operably linked when they are functionally related to each other. For example: a promoter is operably linked to a coding sequence if it controls the transcription of the sequence. Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants.

DNA sequences encoding KAT enzyme may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesiderable joining, and ligation with appropriate ligases. Techniques for such manipulation are disclosed by Maniatis et al, *supra* and are well known in the art.

Expression of the cloned sequence occurs when the expression vector is introduced into an appropriate host cell. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences, for example *E. coli*. Similarly, if an

eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequence. A yeast host may be employed, for example *S. cerevisiae*. Alternatively, insect cells may be used, in which case a baculovirus vector system may be appropriate. Another alternative host is a mammalian cell line, for example COS-1 cells.

The need for control sequences into the expression vector will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Vectors useful for practicing the present invention include plasmids, viruses (including phages), retroviruses, and integrable DNA fragments (i.e., fragments integrable into the host genome by homologous recombinantion). The vectors replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself.

Expression vectors should contain a promoter which is recognized by the host organism. The promoter sequences of the present invention may be either prokaryotic, eukaryotic or viral. Example of suitable prokaryotic sequences include the P_R and P_L promoters of bacteriophage lambda (Hershey, The Bacteriophage Lambda, Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1973); and Hendrix, Lambda II, Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1980)); the *trp*, *rec*A, heat shock, and *lac*Z promoters of *E. coli* and the SV40 early promoter (Benoist et al, Nature, 290:304-310 (1981)).

As far as the Shine-Dalgarno sequence is concerned, preferred examples of suitable regulatory sequences are represented by the Shine-Dalgarno of the replicase gene of the phage MS-2 and of the gene cII of bacteriophage lambda. The Shine-Dalgarno sequence may be directly followed by the DNA encoding KAT and result in the expression of the mature KAT protein.

Alternatively, the DNA encoding KAT may be preceded by a DNA sequence encoding a carrier peptide sequence. In this case, a fusion protein is produced in which the N-terminus of KAT is fused to a carrier peptide, which may help to increase the protein expression levels and intracellular stability, and provide simple means of purification. A preferred carrier peptide includes one or more of the IgG binding domains of Staphylococcus protein A. Fusion proteins comprising IgG binding domains of protein A are easily purified to homogeneity by affinity chromatography, e.g., on IgG-coupled Sepharose. A DNA sequence encoding a recognition site for a proteolytic enzyme such as enterokinase, factor X or procollagenase may immediately precede the sequence for KAT to permit cleavage of the fusion protein to obtain the mature KAT protein.

Moreover, a suitable expression vector includes an appropriate marker which allows the screening of the transformed host cells. The transformation of the selected host is carried out using any one of the various techniques well known to the expert in the art and described in Maniatis et al, *supra*.

One further embodiment of the invention is a prokaryotic host cell transformed with the said expression vector and able to produce, under appropriate culture conditions, the KAT of the invention.

Cultures of cells derived from multicellular organisms are a desiderable host for recombinant KAT synthesis. In principal, any eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture, including insect cells. Propagation of such cells in cell culture has become a routine procedure. See Kruse et al, Tissue Culture, Eds., Academic Press (1973). Examples of useful host cell lines are HeLa cells, CHO and COS cell lines. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate and invertebrate cells are often provided by viral sources. For example, commonly used promoters are derived from Adenovirus 2, polyoma and SV40. See, e.g. U.S. Patent 4,599,308.

An origin of replication may be provided either by construction of the vector to include an exogenous origin or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient.

Rather than using vectors which contain viral origins of replication, one can transform mammalian cells by the method of cotransformation with a selectable marker and the KAT DNA. An example of a suitable marker is dihydrofolate reductase (DHFR) or thymidine kinase. See U.S. Patent No. 4,399,216.

Cloned genes and vectors of the present invention are useful to transform cells which do not ordinarly express KAT to thereafter express this enzyme. Such cells are useful as intermediates for making recombinant KAT preparations useful for drug screening.

Moreover, genes and vectors of the present invention are useful in gene therapy. For such

purposes, adenovirus vectors as well as retroviral vectors as described in Temin et al, U.S. Patent 4,650,764 and Miller, U.S. Patent 4,861,719 may be employed.

Cloned genes of the present invention, and oligonucleotides derived therefrom, are useful for screening for restriction fragment length polymorphism (RFLP) associated with certain disorders.

Oligonucleotides of the present invention are useful as diagnostic tools for probing KAT gene expression in various tissues. For example, tissue can be probed in situ with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques to investigate native expression of this enzyme or pathological conditions relating thereto.

Genetically modified (transfected) cells have been successfully used for cerebral implantation. Cells transfected with the KAT gene can be useful for delivering kynurenic acid (or any other KAT product; see below) to the brain. This may prove to be an attractive means to circumvent the blood-brain barrier for kynurenic acid through peripheral administration of kynurenine (or any appropriate substrate of KAT; see below).

Transfected cells expressing large quantities of KAT are also useful for the production of neuroactive kynurenic analogs. For example, KAT is capable of forming the potent NMDA receptor antagonist and neuroprotectant 7-chlorokynurenic acid from its bioprecursor L-4-chlorokynurenine (J. Med. Chem., 37:334-336 (1994)).

The present invention is explained in greater detail in the following examples. These examples are

intended to be illustrative of the present invention, and should not be constructed as limiting thereof.

EXAMPLE 1
Amino Acid Sequence of Tryptic Fragments of the Rat KAT

Protein Purification

Rat KAT was prepared essentialy as described by Okuno et al, Brain Res., 534:37-44 (1990). The enzyme eluted from a Sephacryl S-200 column was separated by HPLC on a reverse-phase column (SC18, 250 x 4.6 mm, Japan Spectro. Co. Ltd). Elution was performed with a gradient of solvent A (70% vol/vol) acetonitrile in 0.1% trifluoroacetic acid (TFA)) and solvent B (0.1% TFA) applied for 40 min at a flow rate of 1 ml/min.

Trypsin and CNBr Digestion and Fragment Purification

500 pmoles of HPLC-purified rat KAT sample were digested by trypsin as described (Hugli, In: Techniques In Protein Chemistry, Eds., Academic Press, Inc., pages 377-391 (1989)) and by CNBr. These samples were subjected to reverse-phase HPLC after digestion and the resulting peaks collected.

Amino Acid Sequence Analysis

Sequence analysis was performed essentialy as described (Fabbrini et al, FEBS Lett., 286:91-94 (1991)). Figure 1 shows the partial amino acid sequence of rat KAT: N-terminus of mature KAT, (SEQ ID NO:14) a CNBr fragment (SEQ ID NO:15), tryptic fragment 112 of KAT (SEQ ID NO:16) and tryptic fragment 130 of KAT (SEQ ID NO:17).

EXAMPLE 2

Polymerase Chain Reaction (PCR) Cloning

RNA extraction

Total RNA from rat kidney was extracted from small quantities of tissue according to the instruction of RNAzol™ method (RNAzol-Cinna/Biotex Lab., Texas, U.S.A.).

First Strand cDNA Synthesis

First strand cDNA was synthesized from 3mg of total RNA using 2mg oligo polydT (18pb), 4ml of dNTP (2.5mM), 8ml of AMV buffer (TrisHCl pH8.8 250mM/ KCl 200mM/$MgCl_2$ 50mM/ DTT 20mM) in a final volume of 38.75 ml. The solution was boiled for 3 min at 65°C and throw in ice for 10 min; 0.75 ml of RNAsin (40u/ml Promega) and 0.5 ml of AMV Reverse trascriptase (25u/ml Boehringer Mannheim,GmbH, Germany) were added to the cold solution. The reaction was carried on at 42°C for 2h.

Design and Synthesis of Degenerated Oligonucleotides

Since the relative position of tryptic fragments 112 and 130, along the rat KAT primary structure, was unknown four degenerated oligonucleotides 26 bp long were designed and synthesized using a DNA/RNA synthesizer 380B Applied Biosystems. The product of the reaction was purified on Sephadex G50 (Nap 25 Column, Pharmacia).

The sense orientation oligonucleotide, OligoA: (AAYYTNTGYCARCARCAYGAYGTNGT) (SEQ ID NO:20) and the anti-sense orientation oligonucleotide, OligoC: (ACNACRTCRTGYTGYTGRCANARRTT) (SEQ ID NO: 21) based on the peptide sequence Asn-Leu-Cys-Gln-Gln-His-Asp-Val-Val (residues 7-15 of fragment 130 (SEQ ID NO:17)) while the sense

orientation oligonucleotide, OligoB: (ACNGANARRTTYTGRTCXATNCCRTC) (SEQ ID NO:22) and the corresponding anti-sense oligonucleotide, OligoD: (GAYGGNATZGAYCARAAYYTNTCNGT) (SEQ ID NO: 23) based on the peptide sequence Asp-Gly-Ile-Asp-Gln-Asn-Leu-Ser-Val (residues 3-11 of fragment 112 (SEQ ID NO:16)) (N=T/C/A/G; Z=T/C/A; R=A/G; Y=T/C; X=T/G/A) were synthesized.

Polymerase Chain Reaction Condition

The first strand cDNA was divided in two aliquotes and amplified by PCR as described below. The two oligonucleotide, mixture PCR1: oligoA and oligoD and PCR2 OligoB and OligoC were used as primer in the PCR reaction. 70 ng of template cDNA were combined with 10 mg of each set of primers, 10 ml of 10x Taq polymerase buffer (500 mM KCl/100 mM Tris-HCl, pH 8.3), 8 ml of 25mM $MgCl_2$, 8 ml of a dNTP solution (2.5 mM dNTP) and 0.5 ml (2.5 units) of Taq DNA polymerase (Perkin Elmer Cetus). The volume was brought to 100 ml with H_2O and the mixture was overlayed with mineral oil to prevent evaporation. The tube was heated to 94°C for 3 min, denaturation was carried out for 3 minutes at 94°C, annealing for 2 min at 60°C and polymerization for 2 min and 30 seconds at 72°C. The cycle was repeated 30 times.

A specific amplification product was observed only with PCR1. The product of the amplification was a DNA molecule of about 550 bp. The PCR1-amplification product was re-amplified using a new set of oligos, basically with the same sequence of oligoA and oligoC with SalI linkers and 5'-extra nucleotides. OligoE: (GCTAGTCGACACNACRTCRTGYTGYTGRCANARRTT) (SEQ ID NO:24) complementary to nucleotides

coding for peptide 130 (SEQ ID NO:17) and OligoF: (GATCGTCGACGAYGGNATZGAYCARAAYYTNTCNGT) (SEQ ID NO:25) corresponding to nucleotides coding for peptide 112 (SEQ ID NO:16).

After PCR amplification the resulting DNA fragment was digested overnight with the restriction enzyme Sal1 and ligated to the Sal1 site of the cloning plasmid pUC 18 (Yanisch-Perron et al, Gene, 33:103-119 (1985)). The recombinant plasmid was extracted according to the instruction of the Qiagen Plasmid Maxi Protocol; precipitated with PEG and denaturated with NaOH 2N.

Sequencing was carried out with universal and forward primer and subsequently with a series of synthetic oligonucleotide primers according to the dideoxy chain termination method (Sanger et al, Proc. Natl. Acad. Sci. USA, 74:5463-5467 (1977)) using Sequenase (United States Biochemicals Corp., Cleveland, OH).

Both strands of the insert were sequenced revealing an open reading frame of 196 amino acids. Part of the two rat KAT peptides that were sequenced are coded for the corresponding 588 bp open reading frame. This open reading frame is used as probe in the cDNA library screening described in Example 3.

EXAMPLE 3
cDNA Library Screening

About 500,000 recombinant phages of λgt11 rat kidney cDNA library (Clontec Laboratories, USA) were plated on a lawn of *E. coli* Y1090 cells. After an overnight growth at 37°C the recombinant phages were transferred in duplicate nitrocellulose filters, their DNA was denatured, neutralized and baked under vacuum at 80°C for 2h. Prehybridization was carried out at

60°C for 4h in 6xSSC (1X SSC:__), 5x Denhardt's (1X Denhardt:__), 1% SDS, 200 ug/ml salmon sperm DNA. The filters were then hybridized overnight at 60°C in the same mixture with the addition of about 1.5 x 10^6 cpm/ml of labeled probe (see Example 2).

The probe was labeled with (^{32}P) dCTP by Multiprime DNA labelling system (Amersham), purified on Nick Column (Pharmacia) and added to the hybridizing solution.

The filters were washed at 60°C twice in 2xSSC, 0.1% SDS and ones in 1xSSC, 1%SDS. Filters were exposed to Kodak X-AR film (Eastman Kodak Company, Rochester, N.Y., USA) with intensifying screen at -80°C.

Positive phage plaques were isolated and screened again twice in order to isolate single clones.

Recombinant Phage DNA Extraction and Sequencing Methods

About 50,000 phages of each positive clone were plated on a lawn of *E. coli* Y1090 cells. After an overnight growth at 37°C phages were resuspended in SM buffer (100 mM NaCl/8 mM $MgSO_4$/50 mM Tris-HCl, pH 7.5/gelatin 0.001%) and chloroform 0.3%; the suspension was treated with 1 mg of RNAse and 1 mg of DNAse. Phage DNA was precipitated with PEG 10%/1M NaCl, extracted with phenol and phenol:chloroform:iso-amyl alcohol and precipitated with PEG again.

The phage DNA was digested with EcoRI and the insert was ligated to the EcoRI site of pUC18.

The recombinant plasmid was extracted according to the instruction of Qiagen Plasmid Maxi Protocol; precipitated with PEG and denaturated with 2N NaOH.

Sequencing was carried out with universal and forward primer and subsequently with a series of synthetic oligonucleotide primers according to the dideoxy chain termination method (Sanger et al, supra) using Sequenase (United States Biochemicals Corp., Cleveland, OH).

Three positive clones were isolated, cDNA-1, cDNA-2 and cDNA-3. Both strands of the three cDNAs were sequenced (see Figures 2A-2C, 3A-3D and 4A-4D).

cDNA-1 encodes a deduced protein of 423 amino acid residues, cDNA-2 encodes a deduced protein of 437 amino acid residues and cDNA-3 encodes a deduced protein of 457 amino acid residues.

The three deduced proteins differ only in their N-terminus. Moreover, the cDNA-2 and cDNA-3 clones are not homogeneous, since an alternative 5' sequence introduces an upstream ATG starting codon.

As already said, the longer proteins deduced from the cDNA-2 and cDNA-3 clones present a putative mitochondrial transit peptide in position 1 to 24 (cDNA-2) and in position 1 to 44 (cDNA-3) which is only partially present in the 423 amino acid protein.

EXAMPLE 4
Cloning of human KAT

A λ ZapII human brain cDNA library (Stratagene) was screened with a probe representing the N-terminal part of the cDNA-1 encompassing a sequence from amino acid residue 11 to 197 and encoding rat kydney KAT. About 1,350,000 recombinant phages were plated on a lawn of E. coli XL1 blue cells and screening was performed as described in the Example 3.

Positive phage plaques were isolated and screened again twice in order to isolate single clones.

Recombinant Phage DNA Extraction and Sequencing Methods

E. coli XL1 blue cells were coinfected with about 10^5 phage particles corresponding to the positive clone selected and 1 µl of EX Assist helper phage (10^6 pfu/ml). The mixture was incubated at 37°C for 15 min and later incubated with 3 ml of LB for 3 h. Cells were spinned down and the supernatant was heated at 70°C for 15 min SORL cells at OD600=1 were mixed with the supernatant containing the phagemid pBluescript and incubated for 15 min at 37°C and plated on LB-ampicillin plates (50 µg/ml). Single clones were incubated overnight in LB-ampicillin and DNA was extracted according to the instruction of Qiagen Plasmid Maxi Protocol, precipitated with PEG and denaturated with NaOH 2N. Sequencing was carried out with universal and forward primer and subsequently with a series of synthetic oligonucleotide primers according to the dideoxy chain termination method (Sanger et al, *supra*) using Sequenase (United States Biochemicals Corp., Cleveland, OH).

Unfortunately none of the positive clones contained a full length sequence. Therefore, in order to isolate the 5' lacking sequence a RACE protocol was applied.

5' PCR Race

0.5 µg of polyA+ RNA from human brain was reverse transcribed with a primer (5'-CAGGGCCTGGAAGGCTGTGA-3') (SEQ ID NO:6) located at the N-terminal part of the longest cDNA clone isolated from the human brain cDNA library. Reaction was carried out as described in Example 2. 20 µl were precipitated and resuspended in a mixture containing dATP 0.2 mM, buffer tailing (0.1 M potassium cacodylate pH 6.8, 1 mM $CoCl_2$, 100 mM

DTT, 100 μg/ml BSA) and 15 U TdT enzyme (Gibco BRL). After incubation at 37°C for 10 min water was added to a final reaction volume of 250 μl. cDNA was mixed with 25 pmol of oligo (5'-ATAGCCACCAACAGTCACCA-3') (SEQ ID NO:7), 10 p/mol of oligo (5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTT-3') (SEQ ID NO:8) and 25 pmol of oligo (5'-GACTCGAGTCGACATCGA-3') (SEQ ID NO:9), 10 μl of 10x Taq polymerase buffer (500 mM Kcl/100mM Tris.HCl, pH 8.3), 8 μl of 25 mM $MgCl_2$, 8 μl of a dNTP solution (2.5 mM dNTP). The volume was brought to 100 μl with H_2O. The tube was heated at 95°C for 7 min and 0.5 μl (2.5 U) of Taq polymerase (Perkin Elmer Cetus) were added. Annealing was carried out for 2 min at 58°C and polymerization for 2.5 min at 72°C: The cycle was repated 40 times. PCR products were blotted on a nitrocellulose filter and hybridized as described in Example 3 with a oligonucleotide probe (5'-ACCACTGACGAAGATCCTGGCAAGTTTCTTTGGGGAGC-3') (SEQ ID NO:10), based on the known sequence of the partial cDNA human clone. Probe was labelled with ($\gamma^{32}P$) dATP by T4 polynucleotide Kinase (Boehringer) and purified on Nap5 column (Pharmacia). A positive bands (330 bp) and termed 5'-hKAT was re-amplified using oligo with SalI linkers and cloned in pUC18. DNA sequencing was performed on both strands confirming correspondence between the PCR fragment and the lacking 5'-part of the human KAT clone.

EXAMPLE 5

Expression in Mammalian Cells

The expression plasmid encoding rat KAT was constructed as follows: a) To remove the 5' and the 3' untranslated sequences, as well as the putative mitochondrial targeting peptide, PCR amplification was

performed using two specific oligonucleotides with XhoI linkers. The sense orientation oligonucleotide (5'-TGTCCTCGAGACCATGACCAAACGGCTGCAGGCTCGGA-3') (SEQ ID NO:26) begins at +241 of cDNA-1, whereas the antisense-orientation oligonucleotide (5'-GTACCTCGAGTCAGGGTTGGAGCTCTTTCCACTTG-3') (SEQ ID NO:27) complements the sequence starting from the end of the coding sequence. The XhoI-digested fragment, after being controlled by sequencing, was cloned into the XhoI site of pSVL expression vector (Pharmacia Biotechnology).

The expression plasmid encoding human KAT was constructed as follows. In order to join the two cDNA fragments corresponding to the full length sequence of human KAT two different PCR reactions were carried out: a) 5'hKAT was amplified by PCR using two specific oligonucleotides: a sense primer with XhoI linker (TGTCCTCGAGACCATGGCCAAACAGCTC) (SEQ ID NO:11) and as reverse primer (CAGGGCCTGGAAGGCTGTGA) (SEQ ID NO:6) the oligonucleotide used for the reverse transcription of Race (see Example 4); b) the partial cDNA sequence coding for human KAT obtained after cDNA library screening (Example 4) was PCR amplified using two primers flanking the cloning site: sense primer (GTAATACGACTCACTATAGGGC) (SEQ ID NO:12) and reverse primer (TGTCCTCGAGCGCTCTAGAACTAGTGGATC) (SEQ ID NO:13). The two PCR product were were digested with ApaI, linked together, digested XhoI and cloned into the pSVL vector. COS-1 cells were transfected with 10 ug of pSVL-ratKAT plasmid or pSVL-humanKAT by calcium phosphate method (Maniatis et al, *supra*). 72 h after transfection, cells were disrupted by freezing and thawing and after centrifugation the supernatant was used for KAT, glutamine transaminase K and cysteine conjugate β-lyase activities.

EXAMPLE 6
Kynurenine Amino Transferase, Glutamine Amino Transferase K and Cysteine Conjugate β-lyase Activities

Kynurenine transaminase assay

The reaction mixture (100 ul) contained 70 uM pyridoxal phosphate, 5 mM pyruvate, 3mM kynurenine, and KAT sample in 0.17 M potassium phosphate buffer, pH 8.1, and was incubated at 37°C for 30 min and 1 h. Reaction was stopped by adding 20 ul TCA 50% and the precipitate was removed by centrifugation. The supernatant was analyzed by HPLC with a C18 column (Vydac 201TP54, 25x4.6 cmxmm) at 1 ml/min, equilibrated with 5 mM acetic acid, 5% methanol, 0.1% heptane sulfonic acid, pH 3.0, and kynurenic acid was eluted with 50mM acetic acid, 5% methanol, 0.5% heptane sulfonic acid, pH 4.5. Absorbance at 243 nm was measured.

Glutamine Transaminase K Assay

Glutamine transaminase K activity was measured as described by Cooper and Meister (Methods Enzymol., 113:344-349 (1985)).

Cysteine Conjugate β-lyase Assay

The β-lyase assay was a coupled assay as described by Abraham and Cooper (1991). The product of the β-lyase reaction (pyruvate) was assayed by measuring the oxidation of NADH during the transformation of pyruvate to lactate catalyzed by alanine dehydrogenase. The reaction mixture (200 ml in a microtiter plate) contained 2 mM S-(1,2-dichlorovinyl)-L-cysteine (DCVC), 0.5 mM MTB, 0.1 mM PLP and the enzyme in 100 mM Tris buffer pH 8.8, and was incubated at 37°C for 5, 10, 15 min prior the addition of 0.3 mM NADH, 7.3 U/ml alanine

dehydrogenase, and ammonium acetate 0.1 M. Absorbance at 340 nm was measured using a microplate reader (Cerves uv900) and NADH concentration was calculated using a $\varepsilon 1=4200M^{-1}$.

EXAMPLE 7
Amino Acid Sequence of Tryptic
Fragments of the Human KAT

Human KAT was prepared essentialy as described by Baran et al, J. Neurochem., 62:730-738 (1994).

500 pmoles of the purified human KAT sample were digested by trypsin as described (Hugli, In: Techniques in Protein Chem., pages 377-391, Eds., Academic Press, Inc., (1989)). Briefly, human KAT was desalted using SMART system equipped with Fast desalting column equilibrated in 10 mM ammonium bicarbonate. After the chromatography step sample was concentrated to a final volume of % ml. Cysteine residues in the molecule were reduced in 8 M urea, 10 mM DTT at 50°C for 15 min then the alkylation was carried out with 20 mM iodoacetic acid for 15 min at room temperature. After this time the sample solution was diluted to have a final urea concentration of 2 M and the sample was digested overnight with trypsin (Boehringer) with an enzyme: substrate ratio 1:25. Peptides coming from digestion were analyzed by RP-HPLC using a Vydac C18 column and a linear gradient from 5 to 65% eluent B during 60 min, were eluent A was 0.1% trifluoroacetic acid (TFA) in water and eluent B was 0.07% TFA, 95% acetonitrile. Eluted peaks were manually collected, concentrated using a vacuum speedvac (Savant) and then loaded onto a 477 N-terminal protein sequencer (ABI, Perkin Elmer) for protein sequence determination.

Sequence analysis was performed essentialy as described (Fabbrini et al, FEBS Lett., *supra*). Figure 6 shows the amino acid sequence of three peptides of human KAT, namely F11 (SEQ ID NO:2), F13 (SEQ ID NO:3) and F14 (SEQ ID NO:4).

While the invention has been described in detail, and with reference to specific embodiments thereof, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

IN THE CLAIMS:

Claim 1. An isolated DNA sequence encoding a KAT enzyme selected from the group consisting of:

(a) an isolated DNA sequence which encodes rat KAT enzyme;

(b) an isolated DNA sequence which hybridizes to isolated DNA sequences of (a) above and which encodes a mammalian KAT enzyme; and

(c) an isolated DNA sequence differing from the isolated DNA sequences of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a KAT enzyme.

Claim 2. An isolated DNA sequence according to Claim 1 which encodes rat KAT enzyme.

Claim 3. An isolated DNA sequence according to Claim 1 which encodes rat KAT enzyme, which further comprises the sequence of the clone cDNA-1 (SEQ ID NO:18).

Claim 4. An isolated DNA sequence according to Claim 1 which encodes rat KAT enzyme, which further comprises the sequence of the clone cDNA-2 (SEQ ID NO:19).

Claim 5. An isolated DNA sequence according to Claim 1 which encodes rat KAT enzyme, which further comprises the sequence of the clone cDNA-3 (SEQ ID NO:5).

Claim 6. A vector comprising a cloned DNA sequence as defined in any one of Claims 1 to 5.

Claim 7. A vector comprising a cloned DNA sequence as defined in any one of Claims 1 to 5, wherein the vector is a plasmid.

Claim 8. A vector comprising a cloned DNA sequence as defined in any one of Claims 1 to 5, wherein the vector is a virus.

Claim 9. A vector comprising a cloned DNA sequence as defined in any one of Claims 1 to 5, wherein the vector is a retrovirus.

Claim 10. A host cell transformed with a vector comprising a cloned DNA sequence as defined in any one of Claims 1 to 5.

Claim 11. A host cell transformed with a vector comprising a cloned DNA sequence as defined in any one of Claims 1 to 5, wherein the cell is a mammalian cell.

Claim 12. An oligonucleotide probe capable of selectively hybridizing to a DNA sequence comprising a portion of a gene coding for a KAT enzyme.

Claim 13. An oligonucleotide probe capable of selectively hybridizing to a DNA sequence comprising a portion of a gene coding for a KAT enzyme, wherein the probe is capable of serving as a polymerase chain reaction extension primer.

Claim 14. An oligonucleotide probe capable of selectively hybridizing to a DNA sequence comprising a portion of a gene coding for a KAT enzyme, wherein the probe is labelled with a detectable group.

Claim 15. An oligonucleotide probe capable of selectively hybridizing to a DNA sequence comprising a portion of a gene coding for a KAT enzyme, wherein the probe is labelled with a detectable group, wherein the detectable group is a radioactive atom.

Claim 16. An isolated and purified KAT enzyme which is coded for by a DNA sequence according to any one of Claims 1 to 5.

Claim 17. A method of screening drugs comprising transforming cells useful for drug screening with a vector comprising a cloned DNA sequence as defined in any one of Claims 1-5.

Claim 18. A method of screening drugs comprising using the host cell according to any one of Claims 10 or 11 to screen drugs.

Claim 19. A method of gene therapy comprising cloning a vector useful for gene therapy, which comprises a cloned DNA sequence as defined in any one of Claims 1 to 5.

Claim 20. A method of gene therapy comprising using the host cell according to any one of Claims 10 to 11 for gene therapy.

Claim 21. A method of gene therapy comprising using the host cell according to any one of Claims 10 to 11 for cerebral implantation.

Claim 22. A method of gene therapy comprising using the host cell according to any one of Claims 10 to 11 for delivering kynurenic acid to the brain.

Claim 23. A method of gene therapy comprising cloning an adenovirus vector useful for gene therapy, which comprises a cloned DNA sequence as defined in any one of Claims 1 to 5.

Claim 24. A method of gene therapy comprising isolating a DNA sequence as defined in any one of Claims 1 to 5, wherein said DNA sequence is useful for gene therapy.

Claim 25. A method of screening for restriction fragment length polymorphism, comprising cloning a DNA sequence as defined in any one of Claims 1 to 5, wherein said clone is useful for RFLP screening.

Claim 26. A method of screening for RFLP, comprising using an oligonucleotide probe according to any one of Claims 12 to 15 for RFLP screening.

Claim 27. A method of probing for KAT gene expression, comprising using an oligonucleotide probe

according to any one of Claims 12 to 15 to probe for KAT gene expression.

Claim 28. A method of producing neuroactive kynurenic analogs comprising transforming cells useful for producing neuroactive kynurenic analogs with a vector comprising a cloned DNA sequence as defined in any one of Claims 1 to 5.

Claim 29. A method of producing neuroactive kynurenic analogs comprising using the host cell according to any one of Claims 10 to 11 to produce neuroactive kynurenic analogs.

N - terminal

Leu-Gln-Ala-X-X-Leu-Asp-Gly-Ile-Asp-Gln-Asn-
Leu-X-Val-Glu-Phe-Gly-Lys-Thr-X-Glu-Tyr

CNBr fragment

X-X-Leu-Pro-Gly-Ala-Glu-Asp-Gly-Pro-Tyr-Asp-Arg
-Arg-X-Ala

Tryptic fragment 112

Arg-Leu-Asp-Gly-Ile-Asp-Gln-Asn-Leu-Ser-Val-
Glu-Phe-Gly

Tryptic fragment 130

X-Glu-Leu-Glu-Leu-Val-Ala-Asn-Leu-Cys-Gln-Gln-His
Asp-Val-Cys-Ile-Ser-Asp-Glu-Val-Tyr-Gln-Gln-Val-Tyr-
Asp-Leu-Gly-His-Gln

FIG. 1

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          10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
AAACTGACCAAGGAGTATGATCAATCCCGTCCAGCCTCCGAGCCTGCAGCCGTTTGGTCA                60

TGGTGAGCTGCTTCAGCTAACAATTGCACTGACAGTGCTCTTGAGCCAAGTTGCTTCTGG               120

GCGGAAGTAGTCCATCTAGGGCTCGGCCTCTTTAAAGAAACAGACTTCTGCAACCTTGGG               180

ACTACGTTTGGGGTCGCCGGCTATTGGACGGAGCAGCGCAATTGTTAGCTGAAGCAGCTC               240

ACCATGACCAAACGGCTGCAGGCTCGGAGGCTGGACGGGATTGATCAAAACCTCTGGGTG               300
   MetThrLysArgLeuGlnAlaArgArgLeuAspGlyIleAspGlnAsnLeuTrpVal

GAGTTTGGCAAACTGACCAAGGAGTATGACGTCGTGAACTTGGGTCAGGGCTTCCCTGAC               360
GluPheGlyLysLeuThrLysGluTyrAspValValAsnLeuGlyGlnGlyPheProAsp

TTCTCGCCTCCGGACTTTGCAACGCAAGCTTTTCAGCAGGCTACCAGTGGGAACTTCATG               420
PheSerProProAspPheAlaThrGlnAlaPheGlnGlnAlaThrSerGlyAsnPheMet

CTCAACCAGTACACCAGGGCATTTGGTTACCCACCACTGACAAACGTCCTGGCAAGTTTC               480
LeuAsnGlnTyrThrArgAlaPheGlyTyrProProLeuThrAsnValLeuAlaSerPhe

TTTGGCAAGCTGCTGGGACAGGAGATGGACCCACTCACGAATGTGCTGGTGACAGTGGGT               540
PheGlyLysLeuLeuGlyGlnGluMetAspProLeuThrAsnValLeuValThrValGly

GCCTATGGGGCCTTGTTCACAGCCTTTCAGGCCCTGGTGGATGAAGGAGATGAGGTCATC               600
AlaTyrGlyAlaLeuPheThrAlaPheGlnAlaLeuValAspGluGlyAspGluValIle

ATCATGGAACCTGCTTTTGACTGTTATGAACCCATGACAATGATGGCTGGAGGTTGCCCT               660
IleMetGluProAlaPheAspCysTyrGluProMetThrMetMetAlaGlyGlyCysPro

GTGTTCGTGACTCTGAAGCCGAGCCCTGCTCCTAAGGGGAAACTGGGAGCCAGCAATGAT               720
ValPheValThrLeuLysProSerProAlaProLysGlyLysLeuGlyAlaSerAsnAsp

TGGCAACTGGATCCTGCAGAACTGGCCAGCAAGTTCACACCTCGCACCAAGGTCCTGGTC               780
TrpGlnLeuAspProAlaGluLeuAlaSerLysPheThrProArgThrLysValLeuVal

CTCAACACACCCAACAACCCTTTAGGAAAGGTATTCTCTAGGATGGAGCTGGAGCTGGTG               840
LeuAsnThrProAsnAsnProLeuGlyLysValPheSerArgMetGluLeuGluLeuVal

GCTAATCTGTGCCAGCAGCACGATGTCGTGTGCATCTCTGATGAGGTCTACCAGTGGCTG               900
AlaAsnLeuCysGlnGlnHisAspValValCysIleSerAspGluValTyrGlnTrpLeu
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FIG. 2A

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        10        20        30        40        50        60
12345678901234567890123456789012345678901234567890123456789
GTCTATGACGGGCACCAGCACGTCAGCATCGCCAGCCTCCCTGGCATGTGGGATCGGACC                960
ValTyrAspGlyHisGlnHisValSerIleAlaSerLeuProGlyMetTrpAspArgThr

CTGACCATCGGCAGTGCAGGCAAAAGCTTCAGTGCCACTGGCTGGAAGGTGGGCTGGGTC               1020
LeuThrIleGlySerAlaGlyLysSerPheSerAlaThrGlyTrpLysValGlyTrpVal

ATGGGTCCAGATAACATCATGAAGCACCTGAGGACAGTGCACCAGAATTCTATCTTCCAC               1080
MetGlyProAspAsnIleMetLysHisLeuArgThrValHisGlnAsnSerIlePheHis

TGCCCCACCCAGGCCCAGGCTGCAGTAGCCCAGTGCTTTGAGCGGGAGCAGCAACACTTT              1140
CysProThrGlnAlaGlnAlaAlaValAlaGlnCysPheGluArgGluGlnGlnHisPhe

GGACAACCCCAGCAGCTACTTTTTGCAGCTGCCACAGGCCATGGAGCTGAACCGAGACCAC             1200
GlyGlnProSerSerTyrPheLeuGlnLeuProGlnAlaMetGluLeuAsnArgAspHis

ATGATCCGTAGCCTGCAGTCAGTGGGCCTCAAGCTCTGGATCTCCCAGGGGAGCTACTTC              1260
MetIleArgSerLeuGlnSerValGlyLeuLysLeuTrpIleSerGlnGlySerTyrPhe

CTCATTGCAGACATCTCAGACTTCAAGAGCAAGATGCCTGACCTGCCCGGAGCTGAGGAT              1320
LeuIleAlaAspIleSerAspPheLysSerLysMetProAspLeuProGlyAlaGluAsp

GAGCCTTATGACAGACGCTTTGCCAAGTGGATGATCAAAAACATGGGCTTGGTGGGCATC              1380
GluProTyrAspArgArgPheAlaLysTrpMetIleLysAsnMetGlyLeuValGlyIle

CCTGTCTCCACATTCTTCAGTCGGCCCCATCAGAAGGACTTTGACCACTACATCCGATTC              1440
ProValSerThrPhePheSerArgProHisGlnLysAspPheAspHisTyrIleArgPhe

TGTTTTGTCAAGGACAAGGCCACACTCCAGGCCATGGATGAGAGACTGCGCAAGTGGAAA              1500
CysPheValLysAspLysAlaThrLeuGlnAlaMetAspGluArgLeuArgLysTrpLys

GAGCTCCAACCCTGAGGAGGCTGCCCTCAGCCCCACCTCGAACACAGGCCTCAGCTATGC              1560
GluLeuGlnPro

CTTAGCACAGGGATGGCACTGGAGGGCCCAGCTGTGTGACTGCGCATGTTTCCAGAAAAG              1620

AGGCCATGTCTTGGGGGTTGAAGCCATCCTTTCCCAGTGTCCATCTGGACTATTGGGTTG              1680

GGGGCCAGTTCTGGGTCTCAGCCTACTCCTCTGTAGGTTGCCTGTAGGGTTTTGATTGTT              1740

TCTGGCCTCTCTGCCTGGGGCAGGAAAGGGTGGAATATCAGGCCCGGTACCACCTTAGCC              1800
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FIG. 2B

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         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
CTGCCGAGGCTCTGTGGCTTCTCTACATCTTCTCCTGTGACCTCAGGATGTTGCTACTGT  1860

TCCTAATAAAGTTTTAAGTTATTAGGACCCTCA                             1893
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FIG. 2C

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          10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
GGGCGACTCTAGATTTTTTTTTTTTTTTTACCTTCTACCTTTTATTGTCACGTGAACCATG                60

GTCCTACAGGCTGCTGACAAGCTTGGCTGAGCAGGGATCCCAGGGGCGTCGGCAGGAGAT               120

GAGGAAGGGTTGCTGGGAGGGCTTGGCCTCTTCCTTGAGAAGACAGCAAATGTATCCAGC               180

CTAGATTAAGGGTAGGGCATCCCCTATCCCTGTCAGTGGGCCTAGATCTCAGAGCCCCAC               240

ATTAAAGACTGCTAATGGGTCAGAAATGGGGGTCCCTTAGATGGGGGTAGGCAGCAAGGC               300

CCTCCCTCCAGTGTTCTCATTCTGTTCCGGTTTCATTTGTTGTGTCCAGGGACGGTGAAG               360

CAGATACCAGTCTCAAGCCCCAGGGTGCAGGAAGACGGGAAATGGGAAAATGGAAACATT               420

CTTCAAGTGACCAGAGCACTCTGCCGGGGACAAAAGACTTTGCCTTGAACGCGTAGTGGA               480

GAAGCTACAAACCCCAGGTCCCAGTGGCCTGATTGACTTAGGGTCTCAGCTGGCCCAAAA               540

CTCAGTGTGTAGATCAGACTGATCTCAAACTCACAGAGATCTCCCTGCCTTTGCCTGCTG               600

AGTCCTGGGATTAAAGGCATGAATCACAGTACCTGGTGCCTTTTCTTTAAAAAGCTCACC               660
                    MetAsnHisSerThrTrpCysLeuPhePheLysLysLeuThr
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FIG. 3A

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         10        20        30        40        50        60
12345678901234567890123456789012345678901234567890123456789012345678901234567890
ATGACCAAACGGCTGCAGGCTCGGAGGCTGGACGGGATTGATCAAAACCTCTGGGTGGAG                    720
MetThrLysArgLeuGlnAlaArgArgLeuAspGlyIleAspGlnAsnLeuTrpValGlu
MetThrLysArgLeuGlnAlaArgArgLeuAspGlyIleAspGlnAsnLeuTrpValGlu

TTTGGCAAACTGACCAAGGAGTATGACGTCGTGAACTTGGGTCAGGGCTTCCCTGACTTC                    780
PheGlyLysLeuThrLysGluTyrAspValValAsnLeuGlyGlnGlyPheProAspPhe
PheGlyLysLeuThrLysGluTyrAspValValAsnLeuGlyGlnGlyPheProAspPhe

TCGCCTCCGGACTTTGCAACGCAAGCTTTTCAGCAGGCTACCAGTGGGAACTTCATGCTC                    840
SerProProAspPheAlaThrGlnAlaPheGlnGlnAlaThrSerGlyAsnPheMetLeu
SerProProAspPheAlaThrGlnAlaPheGlnGlnAlaThrSerGlyAsnPheMetLeu

AACCAGTACACCAGGGCATTTGGTTACCCACCACTGACAAACGTCCTGGCAAGTTTCTTT                    900
AsnGlnTyrThrArgAlaPheGlyTyrProProLeuThrAsnValLeuAlaSerPhePhe
AsnGlnTyrThrArgAlaPheGlyTyrProProLeuThrAsnValLeuAlaSerPhePhe

GGCAAGCTGCTGGGACAGGAGATGGACCCACTCACGAATGTGCTGGTGACAGTGGGTGCC                    960
GlyLysLeuLeuGlyGlnGluMetAspProLeuThrAsnValLeuValThrValGlyAla
GlyLysLeuLeuGlyGlnGluMetAspProLeuThrAsnValLeuValThrValGlyAla

TATGGGGCCTTGTTCACAGCCTTTCAGGCCCTGGTGGATGAAGGAGATGAGGTCATCATC                   1020
TyrGlyAlaLeuPheThrAlaPheGlnAlaLeuValAspGluGlyAspGluValIleIle
TyrGlyAlaLeuPheThrAlaPheGlnAlaLeuValAspGluGlyAspGluValIleIle

ATGGAACCTGCTTTTGACTGTTATGAACCCATGACAATGATGGCTGGAGGTTGCCCTGTG                   1080
MetGluProAlaPheAspCysTyrGluProMetThrMetMetAlaGlyGlyCysProVal
MetGluProAlaPheAspCysTyrGluProMetThrMetMetAlaGlyGlyCysProVal

TTCGTGACTCTGAAGCCGAGCCCTGCTCCTAAGGGGAAACTGGGAGCCAGCAATGATTGG                   1140
PheValThrLeuLysProSerProAlaProLysGlyLysLeuGlyAlaSerAsnAspTrp
PheValThrLeuLysProSerProAlaProLysGlyLysLeuGlyAlaSerAsnAspTrp

CAACTGGATCCTGCAGAACTGGCCAGCAAGTTCACACCTCGCACCAAGGTCCTGGTCCTC                   1200
GlnLeuAspProAlaGluLeuAlaSerLysPheThrProArgThrLysValLeuValLeu
GlnLeuAspProAlaGluLeuAlaSerLysPheThrProArgThrLysValLeuValLeu

AACACACCCAACAACCCTTTAGGAAAGGTATTCTCTAGGATGGAGCTGGAGCTGGTGGCT                   1260
AsnThrProAsnAsnProLeuGlyLysValPheSerArgMetGluLeuGluLeuValAla
AsnThrProAsnAsnProLeuGlyLysValPheSerArgMetGluLeuGluLeuValAla

AATCTGTGCCAGCAGCACGATGTCGTGTGCATCTCTGATGAGGTCTACCAGTGGCTGGTC                   1320
AsnLeuCysGlnGlnHisAspValValCysIleSerAspGluValTyrGlnTrpLeuVal
AsnLeuCysGlnGlnHisAspValValCysIleSerAspGluValTyrGlnTrpLeuVal
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FIG. 3B

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         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
TATGACGGGCACCAGCACGTCAGCATCGCCAGCCTCCCTGGCATGTGGGATCGGACCCTG                1380
TyrAspGlyHisGlnHisValSerIleAlaSerLeuProGlyMetTrpAspArgThrLeu
TyrAspGlyHisGlnHisValSerIleAlaSerLeuProGlyMetTrpAspArgThrLeu

ACCATCGGCAGTGCAGGCAAAAGCTTCAGTGCCACTGGCTGGAAGGTGGGCTGGGTCATG                1440
ThrIleGlySerAlaGlyLysSerPheSerAlaThrGlyTrpLysValGlyTrpValMet
ThrIleGlySerAlaGlyLysSerPheSerAlaThrGlyTrpLysValGlyTrpValMet

GGTCCAGATAACATCATGAAGCACCTGAGGACAGTGCACCAGAATTCTATCTTCCACTGC                1500
GlyProAspAsnIleMetLysHisLeuArgThrValHisGlnAsnSerIlePheHisCys
GlyProAspAsnIleMetLysHisLeuArgThrValHisGlnAsnSerIlePheHisCys

CCCACCCAGGCCCAGGCTGCAGTAGCCCAGTGCTTTGAGCGGGAGCAGCAACACTTTGGA                1560
ProThrGlnAlaGlnAlaAlaValAlaGlnCysPheGluArgGluGlnGlnHisPheGly
ProThrGlnAlaGlnAlaAlaValAlaGlnCysPheGluArgGluGlnGlnHisPheGly

CAACCCAGCAGCTACTTTTTGCAGCTGCCACAGGCCATGGAGCTGAACCGAGACCACATG                1620
GlnProSerSerTyrPheLeuGlnLeuProGlnAlaMetGluLeuAsnArgAspHisMet
GlnProSerSerTyrPheLeuGlnLeuProGlnAlaMetGluLeuAsnArgAspHisMet

ATCCGTAGCCTGCAGTCAGTGGGCCTCAAGCTCTGGATCTCCCAGGGGAGCTACTTCCTC                1680
IleArgSerLeuGlnSerValGlyLeuLysLeuTrpIleSerGlnGlySerTyrPheLeu
IleArgSerLeuGlnSerValGlyLeuLysLeuTrpIleSerGlnGlySerTyrPheLeu

ATTGCAGACATCTCAGACTTCAAGAGCAAGATGCCTGACCTGCCCGGAGCTGAGGATGAG                1740
IleAlaAspIleSerAspPheLysSerLysMetProAspLeuProGlyAlaGluAspGlu
IleAlaAspIleSerAspPheLysSerLysMetProAspLeuProGlyAlaGluAspGlu

CCTTATGACAGACGCTTTGCCAAGTGGATGATCAAAAACATGGGCTTGGTGGGCATCCCT                1800
ProTyrAspArgArgPheAlaLysTrpMetIleLysAsnMetGlyLeuValGlyIlePro
ProTyrAspArgArgPheAlaLysTrpMetIleLysAsnMetGlyLeuValGlyIlePro

GTCTCCACATTCTTCAGTCGGCCCCATCAGAAGGACTTTGACCACTACATCCGATTCTGT                1860
ValSerThrPhePheSerArgProHisGlnLysAspPheAspHisTyrIleArgPheCys
ValSerThrPhePheSerArgProHisGlnLysAspPheAspHisTyrIleArgPheCys

TTTGTCAAGGACAAGGCCACACTCCAGGCCATGGATGAGAGACTGCGCAAGTGGAAAGAG                1920
PheValLysAspLysAlaThrLeuGlnAlaMetAspGluArgLeuArgLysTrpLysGlu
PheValLysAspLysAlaThrLeuGlnAlaMetAspGluArgLeuArgLysTrpLysGlu

CTCCAACCCCTGAGGAGGCTGCCCTCAGCCCCACCTCGAACACAGGCCTCAGCTATGCCTT              1980
LeuGlnPro
LeuGlnPro
```

FIG. 3C

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
AGCACAGGGATGGCACTGGAGGGCCCAGCTGTGTGACTGCGCATGTTTCCAGAAAAGAGG                2040

CCATGTCTTGGGGGTTGAAGCCATCCTTTCCCAGTGTCCATCTGGACTATTGGGTTGGGG               2100

GCCAGTTCTGGGTCTCAGCCTACTCCTCTGTAGGTTGCCTGTAGGGTTTTGATTGTTTCT               2160

GGCCTCTCTGCCTGGGGCAGGAAAGGGTGGAATATCAGGCCCGGTACCACCTTAGCCCTG               2220

CCGAGGCTCTGTGGCTTCTCTACATCTTCTCCTGTGACCTCAGGATGTTGCTACTGTTCC               2280

TAATAAAGTTTTAAGTTATTAGGA                                                   2304
```

FIG. 3D

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
AAACTGACCAAGGAGTATGATCAATCCCGTCCAGCCTCCGAGCCTGCAGCCGTTTGGTCA      60

TGGTGAGCTGCTTCAGCTAACAATTGCACTGACAGTGCTCTTGAGCCAAGTTGCTTCTGG      120

GCGGAAGTAGTCCATCTAGGGCTCGGCCTCTTTAAAGAAACAGACTTCTGCAACCTTGGG      180

ACTACGTTTGGGGTCGCCGGCTATTGGACGGAGCAGCGCAATTGTTAGCTGAAGCAGAAC      240

TGTGTGTGGACTCAGGCCCTGGCTTGGAGCCATTTTCTGGGCTAGGCTGTCTGCCCTTCT      300

GTCCCTCTGGAGGGGAAGCCTGCAGTGCCTGTGGACCTACCTCAGAGGCATGTTCAGGAG      360
                                                    MetPheArgSe

TGCAGCAGCCCCTCTCGGTGCACCTGATGTGGCCACTCTGGGGAAGGAAAGCTGGAGCCTC      420
rAlaAlaAlaLeuSerValHisLeuMetTrpProLeuTrpGlyArgLysAlaGlyAlaSe

ACTCACCCGGTGCTTGCACCAGTCTCTCACCATGACCAAACGGCTGCAGGCTCGGAGGCT      480
rLeuThrArgCysLeuHisGlnSerLeuThrMetThrLysArgLeuGlnAlaArgArgLe
                                        MetThrLysArgLeuGlnAlaArgArgLe

GGACGGGATTGATCAAAACCTCTGGGTGGAGTTTGGCAAACTGACCAAGGAGTATGACGT      540
uAspGlyIleAspGlnAsnLeuTrpValGluPheGlyLysLeuThrLysGluTyrAspVa
uAspGlyIleAspGlnAsnLeuTrpValGluPheGlyLysLeuThrLysGluTyrAspVa

CGTGAACTTGGGTCAGGGCTTCCCTGACTTCTCGCCTCCGGACTTTGCAACGCAAGCTTT      600
lValAsnLeuGlyGlnGlyPheProAspPheSerProProAspPheAlaThrGlnAlaPh
lValAsnLeuGlyGlnGlyPheProAspPheSerProProAspPheAlaThrGlnAlaPh

TCAGCAGGCTACCAGTGGGAACTTCATGCTCAACCAGTACACCAGGGCATTTGGTTACCC      660
eGlnGlnAlaThrSerGlyAsnPheMetLeuAsnGlnTyrThrArgAlaPheGlyTyrPr
eGlnGlnAlaThrSerGlyAsnPheMetLeuAsnGlnTyrThrArgAlaPheGlyTyrPr
```

FIG. 4A

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
ACCACTGACAAACGTCCTGGCAAGTTTCTTTGGCAAGCTGCTGGGACAGGAGATGGACCC    720
cProLeuThrAsnValLeuAlaSerPhePheGlyLysLeuLeuGlyGlnGluMetAspPr
oProLeuThrAsnValLeuAlaSerPhePheGlyLysLeuLeuGlyGlnGluMetAspPr

ACTCACGAATGTGCTGGTGACAGTGGGTGCCTATGGGGCCTTGTTCACAGCCTTTCAGGC    780
oLeuThrAsnValLeuValThrValGlyAlaTyrGlyAlaLeuPheThrAlaPheGlnAl
oLeuThrAsnValLeuValThrValGlyAlaTyrGlyAlaLeuPheThrAlaPheGlnAl

CCTGGTGGATGAAGGAGATGAGGTCATCATCATGGAACCTGCTTTTGACTGTTATGAACC    840
aLeuValAspGluGlyAspGluValIleIleMetGluProAlaPheAspCysTyrGluPr
aLeuValAspGluGlyAspGluValIleIleMetGluProAlaPheAspCysTyrGluPr

CATGACAATGATGGCTGGAGGTTGCCCTGTGTTCGTGACTCTGAAGCCGAGCCCTGCTCC    900
oMetThrMetMetAlaGlyGlyCysProValPheValThrLeuLysProSerProAlaPr
oMetThrMetMetAlaGlyGlyCysProValPheValThrLeuLysProSerProAlaPr

TAAGGCGAAACTGGGAGCCAGCAATGATTGGCAACTGGATCCTGCAGAACTGGCCAGCAA    960
oLysGlyLysLeuGlyAlaSerAsnAspTrpGlnLeuAspProAlaGluLeuAlaSerLy
oLysGlyLysLeuGlyAlaSerAsnAspTrpGlnLeuAspProAlaGluLeuAlaSerLy

GTTCACACCTCGCACCAAGGTCCTGGTCCTCAACACACCCAACAACCCTTTAGGAAAGGT   1020
sPheThrProArgThrLysValLeuValLeuAsnThrProAsnAsnProLeuGlyLysVa
sPheThrProArgThrLysValLeuValLeuAsnThrProAsnAsnProLeuGlyLysVa

ATTCTCTAGGATGGAGCTGGAGCTGGTGGCTAATCTGTGCCAGCAGCACGATGTCGTGTG   1080
lPheSerArgMetGluLeuGluLeuValAlaAsnLeuCysGlnGlnHisAspValValCy
lPheSerArgMetGluLeuGluLeuValAlaAsnLeuCysGlnGlnHisAspValValCy

CATCTCTGATGAGGTCTACCAGTGGCTGGTCTATGACGGGCACCAGCACGTCAGCATCGC   1140
sIleSerAspGluValTyrGlnTrpLeuValTyrAspGlyHisGlnHisValSerIleAl
sIleSerAspGluValTyrGlnTrpLeuValTyrAspGlyHisGlnHisValSerIleAl

CAGCCTCCCTGGCATGTGGGATCGGACCCTGACCATCGGCAGTGCAGGCAAAAGCTTCAG   1200
aSerLeuProGlyMetTrpAspArgThrLeuThrIleGlySerAlaGlyLysSerPheSe
aSerLeuProGlyMetTrpAspArgThrLeuThrIleGlySerAlaGlyLysSerPheSe

TGCCACTGGCTGGAAGGTGGGCTGGGTCATGGGTCCAGATAACATCATGAAGCACCTGAG   1260
rAlaThrGlyTrpLysValGlyTrpValMetGlyProAspAsnIleMetLysHisLeuAr
rAlaThrGlyTrpLysValGlyTrpValMetGlyProAspAsnIleMetLysHisLeuAr

GACAGTGCACCAGAATTCTATCTTCCACTGCCCCACCCAGGCCCAGGCTGCAGTAGCCCA   1320
gThrValHisGlnAsnSerIlePheHisCysProThrGlnAlaGlnAlaAlaValAlaGl
gThrValHisGlnAsnSerIlePheHisCysProThrGlnAlaGlnAlaAlaValAlaGl
```

FIG. 4B

```
        10        20        30        40        50        60
12345678901234567890123456789012345678901234567890123456789
GTGCTTTGAGCGGGAGCAGCAACACTTTGGACAACCCAGCAGCTACTTTTTGCAGCTGCC 1380
nCysPheGluArgGluGlnGlnHisPheGlyGlnProSerSerTyrPheLeuGlnLeuPr
nCysPheGluArgGluGlnGlnHisPheGlyGlnProSerSerTyrPheLeuGlnLeuPr

ACAGGCCATGGAGCTGAACCGAGACCACATGATCCGTAGCCTGCAGTCAGTGGGCCTCAA 1440
oGlnAlaMetGluLeuAsnArgAspHisMetIleArgSerLeuGlnSerValGlyLeuLy
oGlnAlaMetGluLeuAsnArgAspHisMetIleArgSerLeuGlnSerValGlyLeuLy

GCTCTGGATCTCCCCAGGGGAGCTACTTCCTCATTGCAGACATCTCAGACTTCAAGAGCAA 1500
sLeuTrpIleSerGlnGlySerTyrPheLeuIleAlaAspIleSerAspPheLysSerLy
sLeuTrpIleSerGlnGlySerTyrPheLeuIleAlaAspIleSerAspPheLysSerLy

GATGCCTGACCTGCCCCGGAGCTGAGGATGAGCCTTATGACAGACGCTTTGCCAAGTGGAT 1560
sMetProAspLeuProGlyAlaGluAspGluProTyrAspArgArgPheAlaLysTrpMe
sMetProAspLeuProGlyAlaGluAspGluProTyrAspArgArgPheAlaLysTrpMe

GATCAAAAACATGGGCTTGGTGGGCATCCCTGTCTCCACATTCTTCAGTCGGCCCCATCA 1620
tIleLysAsnMetGlyLeuValGlyIleProValSerThrPhePheSerArgProHisGl
tIleLysAsnMetGlyLeuValGlyIleProValSerThrPhePheSerArgProHisGl

GAAGGACTTTGACCACTACATCCGATTCTGTTTTGTCAAGGACAAGGCCACACTCCAGGC 1680
nLysAspPheAspHisTyrIleArgPheCysPheValLysAspLysAlaThrLeuGlnAl
nLysAspPheAspHisTyrIleArgPheCysPheValLysAspLysAlaThrLeuGlnAl

CATGGATGAGAGACTGCGCAAGTGGAAAGAGCTCCAACCCTGAGGAGGCTGCCCTCAGCC 1740
aMetAspGluArgLeuArgLysTrpLysGluLeuGlnPro
aMetAspGluArgLeuArgLysTrpLysGluLeuGlnPro

CCACCTCGAACACAGGCCTCAGCTATGCCTTAGCACAGGGATGGCACTGGAGGGCCCAGC 1800

TGTGTGACTGCGCATGTTTCCAGAAAAGAGGCCATGTCTTGGGGGTTGAAGCCATCCTTT 1860

CCCAGTGTCCATCTGGACTATTGGGTTGGGGGCCAGTTCTGGGTCTCAGCCTACTCCTCT 1920

GTAGGTTGCCTGTAGGGTTTTGATTGTTTCTGGCCTCTCTGCCTGGGGCAGGAAAGGGTG 1980
```

FIG. 4C

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
GAATATCAGGCCCGGTACCACCTTAGCCCTGCCGAGGCTCTGTGGCTTCTCTACATCTTC          2040

TCCTGTGACCTCAGGATGTTGCTACTGTTCCTAATAAAGTTTTAAGTTATTAGGACCCTC          2100

C                                                                      2101
```

FIG. 4D

FIG. 5A

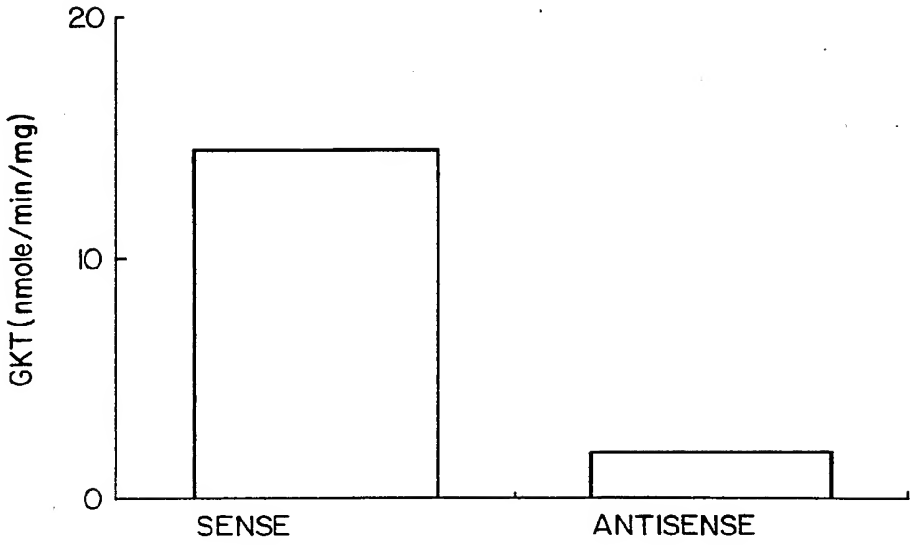
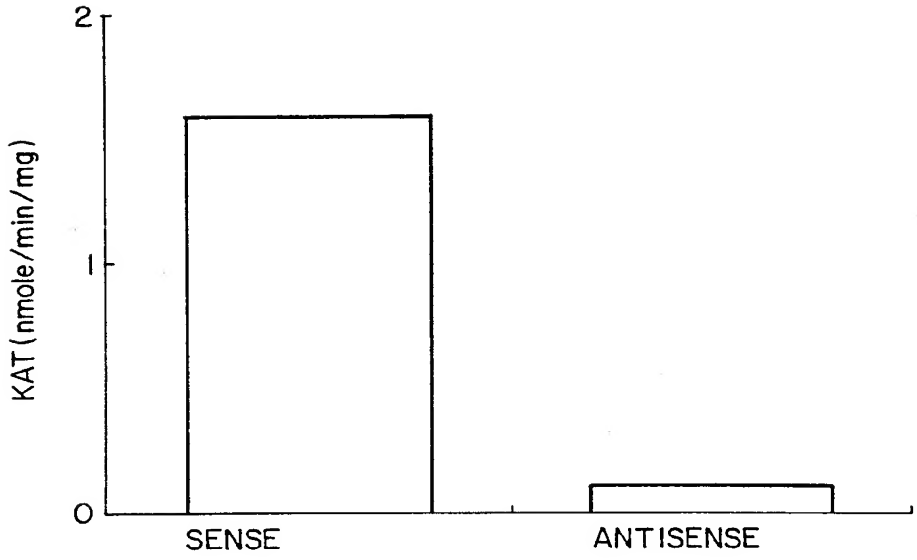

20
10
0
GKT (nmole/min/mg)
SENSE
ANTISENSE

FIG. 5B


2
1
0
KAT (nmole/min/mg)
SENSE
ANTISENSE

Tryptic fragment F11

Thr-Phe-Ser-Ala-Thr-Gly-XXX-Lys

The sequence corresponds to positions 191-198 of the protein deduced from the human brain cDNA sequence

Tryptic fragment F13

Ala-Leu-Val-Leu-Asn-Thr-Pro-Asn-Asn-Pro-Leu-Gly-Lys

The sequence corresponds to positions 120-132 of the protein deduced from the human brain cDNA sequence

Tryptic fragment F14

Glu-Gln-Leu-Leu-Phe-Arg

The sequence corresponds to positions 238-243 of the protein deduced from the human brain cDNA sequence

FIG. 6

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
CTTAATGTTTTTAGAGCTCACCATGGCCAAACAGCTGCAGGCCCGAAGGCTAGACGGGAT                60
                          MetAlaLysGlnLeuGlnAlaArgArgLeuAspGlyIl

CGACTACAACCCCTGGGTGGAGTTTGTGAAACTGGCCAGTGAGCATGACGTCGTGAACTT               120
eAspTyrAsnProTrpValGluPheValLysLeuAlaSerGluHisAspValValAsnLe

GGGCCAGGGCTTCCCGGATTTCCCACCACCAGACTTTGCCGTGGAAGCCTTTCAGCACGC               180
uGlyGlnGlyPheProAspPheProProProAspPheAlaValGluAlaPheGlnHisAl

TGTCAGTGGAGACTTCATGCTTAACCAGTACACCAAGACATTTGGTTACCCACCACTGAC               240
aValSerGlyAspPheMetLeuAsnGlnTyrThrLysThrPheGlyTyrProProLeuTh

GAAGATCCTGGCAAGTTTCTTTGGGGAGCTGCTGGGTCAGGAGATAGACCCGCTCAGGAA              300
rLysIleLeuAlaSerPhePheGlyGluLeuLeuGlyGlnGluIleAspProLeuArgAs

TGTGCTGGTGACTGTTGGTGGCTATGGGGCCCTGTTCACAGCCTTCCAGGCCCTGGTGGA              360
nValLeuValThrValGlyGlyTyrGlyAlaLeuPheThrAlaPheGlnAlaLeuValAs

CGAAGGAGACGAGGTCATCATCATCGAACCCTTTTTTGACTGCTACGAGCCCATGACAAT              420
pGluGlyAspGluValIleIleIleGluProPhePheAspCysTyrGluProMetThrMe

GATGGCAGGGGGTCGTCCTGTGTTTGTGTCCCTGAAGCCGGGTCCCATCCAGAATGGAGA              480
tMetAlaGlyGlyArgProValPheValSerLeuLysProGlyProIleGlnAsnGlyGl

ACTGGGTTCCAGCAGCAACTGGCAGCTGGACCCCATGGAGCTGGCCGGCAAATTCACATC              540
uLeuGlySerSerSerAsnTrpGlnLeuAspProMetGluLeuAlaGlyLysPheThrSe

ACGCACCAAAGCCCTGGTCCTCAACACCCCCAACAACCCCCTGGGCAAGGTGTTCTCCAG              600
rArgThrLysAlaLeuValLeuAsnThrProAsnAsnProLeuGlyLysValPheSerAr

GGAAGAGCTGGAGCTGGTGGCCAGCCTTTGCCAGCAGCATGACGTGGTGTGTATCACTGA              660
gGluGluLeuGluLeuValAlaSerLeuCysGlnGlnHisAspValValCysIleThrAs
```

FIG. 7A

```
        10         20         30         40         50         60
1234567890123456789012345678901234567890123456789012345678901234567890
TGAAGTCTACCAGTGGATGGTCTACGACGGGCACCAGCACATCAGCATTGCCAGCCTCCC                720
pGluValTyrGlnTrpMetValTyrAspGlyHisGlnHisIleSerIleAlaSerLeuPr

TGGCATGTGGGAACGGACCCTGACCATCGGCAGCGCCGGCAAGACCTTCAGCGCCACTGG               780
oGlyMetTrpGluArgThrLeuThrIleGlySerAlaGlyLysThrPheSerAlaThrGl

CTGGAAGGTGGGCTGGGTCCTGGGTCCAGATCACATCATGAAGCACCTGCGGACCGTGCA               840
yTrpLysValGlyTrpValLeuGlyProAspHisIleMetLysHisLeuArgThrValHi

CCAGAACTCCGTCTTCCACTGCCCCACGCAGAGCCAGGCTGCAGTAGCCGAGAGCTTTGA               900
sGlnAsnSerValPheHisCysProThrGlnSerGlnAlaAlaValAlaGluSerPheGl

ACGGGAGCAGCTGCTCTTCCGCCAACCCAGCAGCTACTTTGTGCAGTTCCCGCAGGCCAT               960
uArgGluGlnLeuLeuPheArgGlnProSerSerTyrPheValGlnPheProGlnAlaMe

GCAGCGCTGCCGTGACCACATGATACGTAGCCTACAGTCAGTGGGCCTGAAGCCCATCAT              1020
tGlnArgCysArgAspHisMetIleArgSerLeuGlnSerValGlyLeuLysProIleIl

CCCTCAGGGCAGCTACTTCCTCATCACAGACATCTCAGACTTCAAGAGGAAGATGCCTGA              1080
eProGlnGlySerTyrPheLeuIleThrAspIleSerAspPheLysArgLysMetProAs

CTTGCCTGGAGCTGTGGATGAGCCCTATGACAGACGCTTCGTCAAGTGGATGATCAAGAA              1140
pLeuProGlyAlaValAspGluProTyrAspArgArgPheValLysTrpMetIleLysAs

CAAGGGCTTGGTGGCCATCCCTGTCTCCATCTTCTATAGTGTGCCACATCAGAAGCACTT              1200
nLysGlyLeuValAlaIleProValSerIlePheTyrSerValProHisGlnLysHisPh

TGACCACTATATCCGCTTCTGTTTTGTGAAGGATGAAGCCACGCTCCAGGCCATGGACGA              1260
eAspHisTyrIleArgPheCysPheValLysAspGluAlaThrLeuGlnAlaMetAspGl

GAAGCTGCGGAAGTGGAAGGTGGAACTCTAGCCCTGAAGTCACGCCTTGGCCCTGACATC              1320
uLysLeuArgLysTrpLysValGluLeu...
```

FIG. 7B

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
CCCACATGCCCGCAGAGATCCTCTTTGAGTGTCTGTCTTTGTCCAGGTTTCAGACATTTC  1380

TAGGTTGGGGAAGATGCTATTGGGAAACCTCTTCTCCGTGACACAGAATGTTCTGGGTGG  1440

GAGCCGCCCTTCTTCATCTTAGAGAACCAAGTACCTCCTGTCTGAAAGGTGAGGGTGGCC  1500

TGACCTGGGCCTCTCCCTGCCCCTCCATAGGTGGGTTTGTAGGGTCTTGTGTTGCTTCTG  1560

GTCTCTCCAGGCTTGGCTGAGACGGACGGTAGACTTCCACCATGTACCGATCACATCCCA  1620

ACTCTGCATGGCCCCTGCTAAGGCTCAGGTATAACCTCACCTTCCCTGGCTCATCTTGGC  1680

CTTGGGGAGTTGCCTTTAGGCTTGAGTCCTCAAGCCTCTCCTTTTCGTCCATAATAAAAT  1740

GGGAATTC                                                       1748
```

FIG. 7C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07855

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 9/10, 9/88, 15/54, 15/60, 15/70; C12Q 1/68; A61K 31/70
US CL :435/6, 193, 232, 252.3, 320.1; 536/23.2; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 193, 232, 252.3, 320.1; 536/23.2; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG
search terms: KAT, kynurenine aminotransferase, rat

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	JOURNAL OF BIOCHEM, Vol. 279, issued 1991, MAWAL ET AL., "Purification and properties of kynurenine aminotransferase from rat kidney", PAGES 595-599, SEE ENTIRE DOCUMENT.	16 --- 1-15, 17-29
X --- A	BIOCHIMICA ET BIOPHYSICA ACTA, Vol. 743, issued 1983, TAKEUCHI ET AL, "Purification, characterization and identification of rat liver mitochondrial kynurenine aminotransferasewithalpha-aminoadipateaminotransferase", PAGES 323-330, SEE ENTIRE DOCUMENT	16 --- 1-15, 17-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier document published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&" document member of the same patent family	

Date of the actual completion of the international search
10 OCTOBER 1995

Date of mailing of the international search report
01 NOV 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07855

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07855

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.
Group I, claims 1-11, 17-18 and 28-29, drawn to DNA sequences and first method of use in screening assay.

Group II, claims 12-15, drawn to oligonucleotide probes.

Group III, claim 16, drawn to a KAT enzyme.

Group IV, claims 19-24, drawn to a method of gene therapy.

Group V, claims 25-27, drawn to a method of screening for RFLP's.

The inventions listed as Groups I, and IV or V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I comprises DNA and its first method of use. The subsequent groups IV and V comprise separate methods of use of the DNA compound. These methods of use are unrelated to each other and to the first method of use in their technique and/or additional components, and thus do not relate to a single inventive concept.

The inventions listed as Groups III, and I-II or IV-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The enzyme of Group III is chemically and physically separate from the DNA of Groups I and II, and thus also the methods of using this in Groups IV-V. Thus, the inventions of Groups I-II and IV-V lack a corresponding special technical feature with the enzyme of Group III.

The inventions listed as Groups II, and I or IV-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the follow ingreasons: Group II is directed to oligonucleotide probes, while Group I is directed to whole DNA encoding the enzyme, which has a different activity and use from the oligonucleotides. Thus, Groups IV-V lack the same corresponding special technical feature as well as Group I, and do not relate to Group II.